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US ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE
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# EVALUATION OF EUTHYMIC HAIRLESS GUINEA PIGS [Crl:IAF(HA)BR] AS AN ANIMAL MODEL FOR VESICANT INJURY



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Euthymic hairless guinea pigs [Crl:IAF(HA)BR] were compared to normal haired guinea pigs [Crl:(HA)BR] to determine whether the hairless guinea pig is a suitable animal model for studying sulfur mustard (HD) induced vesication of skin. Neat HD was applied to the skin for 30 minutes, and the exposed skin was examined at 24 and/or 48 hours post-exposure for gross, histopathologic, and/or ultrastructural lesions. The hairless guinea pig proved to be a superior animal model compared to the haired guinea pig because it was more sensitive to HD, the lesions were more readily scored, and the animal was much more convenient to use.


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## PREFACE

The work described in this report is authorized under the US Army Medical Research Institute of Chemical Defense (USAMRICD) animal use protocol number 1-20-88-000-B-497, "Evaluation of Euthymic Hairless Guinea Pigs [Crl:IAF(HA)BR] as an Animal Model for Vesicant Injury." The experimental results/data are recorded in USAMRICD laboratory notebook number 064-88.


## ACKNOWLEDGEMENTS

The authors wish to thank Mr. Floyd Brinkley for his assistance in recording data during the HD challenge phase of the study, the technical staffs of the Comparative Pathology Branch's histology and electron microscopy laboratories for their work in processing the tissue specimens, and Mrs. Connie Clark for her assistance in preparing the figures used in this technical report.

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## INTRODUCTION

Research efforts at the USAMRICD to elucidate the mechanism of action, develop pretreatments and/or develops therapies for sulfur mustard (HD) skin toxicity have been hampered by the lack of a suitable experimental animal model. A literature review and discussion with institute investigators revealed that there is not a definitive animal model that develops elevated blisters/vesicles like those seen in human skin following exposure to sulfur mustard (HD). Re-epithelialized thermally burned guinea pig skin, bird skin, dog mammary gland skin, frog skin, and rabbit ear skin have been reported to form vesicles or vesicle-like structures following applications of HD.[7] Haire guinea pigs have been used to study vesicant injury but blister formation has not been reported.[1,3] It has been recently demonstrated that HD and lewisite form microblisters in swine.[6] Investigators working during World War II speculated that animal skin does not blister like human skin because of (1) th increased number of hair follicles and/or hairs in animal skin, (2) the decreased number of sweat glands in animal skin, and/or (3) the decreased relative thickness of animal skin.[7] It is currently thought that fluid-filled blisters will not form unless (1) there is increased fluid accumulation/pressure resulting from the release of osmotically active tissue breakdown products following tissue injury; (2) the corneum and dermal layers are capable of retaining this fluid; and (3) there is a lack of intradermal structures (i.e., hair roots and/or follicles) which would otherwise prevent the accumulated fluid from separating the dermis from the epidermis.[6] Euthymi hairless guinea pigs [Crl:IAF(HA)BR],[a] a mutant strain that has only recently become commercially available, are basically devoid of hair, have a thickened epidermis when compared with normal haired guinea pigs, and have been shown tc be equal, if not superior, to normal haired guinea pigs for contact sensitivit testing in that they manifest more uniform and superficial skin lesions.[9]

This study was conducted to evaluate the euthymic hairless guinea pig [Crl:IAF(HA)BR] as an animal model for HD skin toxicity compared to the normal haired guinea pig [Crl:(HA)BR].[b]

## MATERIALS AND METHODS

### EXPERIMENTAL DESIGN:

Nineteen male guinea pigs (8 haired & 11 hairless) were divided into thre experimental groups (see Table 1).

Groups I & II: Identical doses of neat HD were applied to the skin of haired and hairless guinea pigs in groups I & II. The exposed skin was examined at 24 and 48 hours post-exposure respectively for the presence of

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[a] Charles River Laboratories, Wilmington, MA 01887, U.S.A.

[b] Charles River Laboratories, Wilmington, MA 01887, U.S.A.

gross, microscopic, and ultrastructural pathology. The unexposed skin from each animal served as the negative control.

Group III: Identical low doses of neat HD were applied to the skin of hairless guinea pigs. The exposed skin was examined at 24 hours post-exposure for the presence of gross, microscopic, and ultrastructural pathology.

TECHNICAL METHODS:

Animal Husbandry: Animals were maintained at 75-80° F. and 50% relative humidity. They are on a 12-hour diurnal light cycle. Food and water were provided ad libitum. Animals were housed in individual polycarbonate shoe box cages with wood shavings prior to exposure to HD. Animals were housed in wire shoe box cages lined with an absorbent plastic-backed paper pad in a chemical fume hood during the post-exposure holding period.

Anesthesia/Analgesia: Guinea pigs were anesthetized with Ketamine HCL[c] (30 mg/kg) and Xylazine[d] (6 mg/kg) during the exposure and decontamination phases of the experiment. The ketamine and xylaxine were injected separately into the left and right lateral thigh muscles using tuberculin syringes and 26 gauge needles.

Methods of Restraint: Guinea pigs were manually restrained while they were being clipped and anesthetized. Anesthetized animals were secured with adhesive tape to a restraining board in sternal recumbency for the exposure and decontamination phases of the experiment.

Surgical Procedures: None.

Animal Preparation: On the day before the guinea pigs were scheduled to be dosed with HD, all animals were assigned an identification number and weighed to the nearest gram. The backs of the haired guinea pigs were clipped with a #40 clipper blade[e] and wiped down with isopropyl alcohol. The backs of the hairless guinea pigs were also wiped with isopropyl alcohol. On the day of HD exposure the animals were anesthetized, exposure site reference points were marked on the skin with a black Sharpie™ permanent marker, and the animals were

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[c] Vetalar™, 100 mg/ml. Parke-Davis, Division of Warner-Lambert Company, Morris Plains, NJ 07950, U.S.A.

[d] Rompun™, 20 mg/ml. Mobay Corporation, Animal Health Division, Shawnee, KS 66201, U.S.A.

[e] Oster™ Golden A-5 Clipper. Oster Professional Products Department, Milwaukee, WI 53217, U.S.A.

covered with a fenestrated (5 cm x 12 cm) plastic barrier drape[f] taped in place so that only exposure sites on the animal backs remained exposed. The 8 exposure sites per animal were arranged in two parallel rows that were 1 cm to the left and right of the dorsal midline and had 2 cm spacing between sites (see Fig. 1).

HD Dosing: All HD dosing, post-exposure handling, and decontamination of animals and materials were performed IAW USAMRICD SOP No. 88-180-DA-13. "Cutaneous Applications of Sulfur Mustard (HD) on Guinea Pigs." Neat (liquid) HD was applied to the skin with either a 1.0 $\mu l$[g] or a 100 $\mu l$[h] syringe. Groups I & II animals had 4 doses of HD (0.5, 1.0, 2.0, & 4.0 $\mu l$) applied to the 4 exposure sites on the left side of the dorsal midline (1 site/dose/animal) and no agent applied to the four (4) contralateral negative control sites to the right of the dorsal midline. Group III animals had 4 doses of agent (0.05, 0.1, 0.2, & 0.4 $\mu l$) applied to the all 8 exposure sites (2 sites/dose/animal). The doses of HD were systematically rotated among the skin exposure sites to control for differences in skin thickness for all groups (see Table 2). The HD was allowed to remain in contact with the skin for 30 minutes for all groups. Following the 30 minute HD skin exposure the animals were placed in holding cages in the hood, allowed to recover from anesthesia, and held for 24 or 48 hours. Sample HD dosing worksheets are attached as Appendix A.

Euthanasia: Animals were euthanatized at the end of the 24 or 48 hour post-exposure holding period with a halothane[i] overdose.[10]

Scoring of Gross Lesions: All sites were scored for lesion diameter, erythema and/or eschar (E/E) formation, and edema and/or blister (E/B) formation at either 24 hours (Groups I & III) or 48 hours (Group II) using a modified method for testing primary irritant substances.[11] The lesion diameter was measured to the nearest millimeter (mm) from the outermost edge of visible erythema. The lesion size for irregularly shaped lesions was the average of that lesion's longest and shortest dimension. The degree of E/E and E/B formation was scored on a scale of 0-4. Sample gross lesion worksheets are attached as Appendix B.

Light Microscopy: Following euthanasia and scoring of gross lesions the skin on the backs of the animals was excised using a #15 scalpel blade, taking care to include and not to traumatize all 8 skin sites/animal. The skin

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[f] 3M Steri-Drape[T], Style No. 1020. 3M Company, Medical Products Division, St. Paul, MN 55119, U.S.A.

[g] Hamilton[T] Model 7001N Microliter Syringe. Hamilton Company, Reno, NV 89502, U.S.A.

[h] Hamilton[T] Model 710N Microliter Syringe. Hamilton Company, Reno, NV 89502, U.S.A.

[i] Halothane, U.S.P. Halocarbon Laboratories, Inc., Hackensack, NJ 07601, U.S.A.

specimens were immersed in a 4% formaldehyde:1% glutaraldehyde (4CF:1G) fixative[5] for 2-3 hours. The lesions were then cut in half and the lateral 1/2 of each skin site was placed in 10% neutral buffered formalin (NBF) for at least 24 hours prior to trimming and processing the tissue for examination by light microscopy. The remaining medial 1/2 of each skin site was kept in 4CF:1G for electron microscopy (EM). All NBF fixed tissue specimens were embedded in glycol methacrylate (epon plastic) or paraffin, cut into 4 micrometer ($\mu m$) thick sections, and stained with hematoxylin and eosin (H&E) using standard histology techniques. The extent of coagulative necrosis and the presence or absence of pathologic changes were scored for each specimen using the sample histopathology worksheet attached as Appendix C. The scoring of the lesions and the selection of tissue specimens for EM were done without knowledge of the dose of HD that had been applied (i.e., a "blind" control).

Electron Microscopy: Four skin specimens from skin exposure sites showing the best vesicle/cleft formation on light microscopy were submitted for scanning and transmission EM. Areas examined included centers of blisters and junctions of blistered and adjacent nonblistered skin. The 4CF:1G fixed tissues selected for transmission EM were transferred to a fresh solution of 4CF:1G and held at 4° C. for at least 48 hours. The tissues were then washed in buffer, post-fixed in 1% buffered osmium tetroxide, dehydrated in graded ethanols, embedded in epoxy resin, cut into 1 $\mu m$ semithin sections, stained with Humphrey's stain[4] and examined by light microscopy to select appropriate areas for thin section analysis. Areas identified were cut into 1,000 Å thin sections and counterstained with lead citrate and uranyl acetate. Tissues selected for scanning EM were washed in buffer, dehydrated in graded ethanols, critical point dried, and sputter-coated with gold-palladium.

Data Analysis: The average lesion size, erythema/eschar score, and edema/blister score for each HD dose and observation time in the hairless guinea pig were compared to the corresponding haired counterpart. Lesion size data was analyzed using the Student's t-test ($H_o$: $\mu_1 = \mu_2$; $\alpha = 0.05$; $df = n_1 + n_2 - 2$).[2] Erythema/eschar and edema/blister data was analyzed using the Mann-Whitney Test ($H_o$: $M_1 = M_2$; $\alpha = 0.05$).[2]

## RESULTS

### CLINICAL OBSERVATIONS:

All animals tolerated the anesthetic regimen and restraint without complications. The hairless guinea pigs appeared to be much more sensitive to the HD than were the haired guinea pigs as evidenced by the markedly visible erythema which developed within 7-30 minutes following the application of HD. The application of neat HD to the skin also appeared to cause some degree of pruritus because numerous Group I & II animals scratched at the exposure sites within 3-5 hours following the application of HD to the point of causing self-inflicted breaks in the skin which complicated the scoring of gross lesions at the end of the 24 and 48 hour post-exposure holding periods. To alleviate this apparent pruritus and to minimize self-inflicted trauma, Group III animals

received a second dose of ketamine and xylazine 3 hours following the HD challenge.

GROSS PATHOLOGY:

All skin exposures to HD resulted in gross skin lesions consisting of well-defined, irregularly shaped, and moderately elevated areas of swelling and erythema, whereas none of the negative control skin sites developed lesions (see Figure 2). At 24 hours post-exposure the size of the skin lesions ranged from 0-14 mm in haired animals and from 2-22 mm in hairless animals. At 48 hours post-exposure the size of the skin lesions ranged from 5-16 mm in haired animals and from 8-24 mm in hairless animals. The lesion diameter increased with both dose and/or time in both the haired and hairless guinea pigs with the hairless guinea pigs having significantly larger lesions at corresponding doses at 24 hours post-exposure. There was no statistically significant difference in lesion size between the haired and hairless guinea pigs for any of the doses at 48 hours post-exposure (see Tables 3-4 and Figures 3-4). The erythema/eschar scores at 24 hours post-exposure ranged from 0-4 in haired animals and from 1-4 in hairless animals. At 48 hours post-exposure the erythema/eschar scores ranged from 1-4 in haired animals and from 2-4 in hairless animals. In haired guinea pigs, scab formation occurred in 31.25% of the exposure sites at the 0.5-4.0 µl HD dosage level. In hairless animals, scab formation occurred in 59.38% of the exposure sites at the 0.5-4.0 µl HD dosage level and in 58.33% of the exposure sites at the 0.05-0.4 µl HD dosage level. The erythema/eschar scores increased slightly or not at all with increases in dose and/or time in both the haired and hairless guinea pigs with the hairless guinea pigs having slightly higher scores at corresponding dose and time points (see Tables 3-4 and Figures 5-6). The edema/blister scores at 24 hours post-exposure ranged from 0-2 in haired animals and from 0-3 in hairless animals. At 48 hours post-exposure the edema/blister scores ranged from 1-2 in haired animals and from 1-3 in hairless animals. The edema/blister scores increased slightly or not at all with increases in dose and/or time in both the haired and hairless guinea pigs with the hairless guinea pigs having slightly higher scores at corresponding dose and time points (see Tables 3-4 and Figures 7-8).

LIGHT MICROSCOPY:

All skin exposures to HD resulted in skin lesions. The skin lesions typically consisted of coagulation necrosis of the epidermis and superficial dermis wherever HD had direct skin contact. The width of the necrotic areas (0.5-2.0 cm) was directly proportional to the dose of HD. Histologically, the necrosis involved the entire thickness of the epidermis and extended into the superficial dermal collagen (i.e., a total depth of 1-2 mm). Haired guinea pig skin reacted less severely to identical doses of HD. Microblisters and less severe inflammatory changes developed within the epidermis adjacent to the coagulation necrosis with microblister formation being more prevalent at lower HD doses. The morphologic changes seen in these areas consisted of ballooning degeneration and separation of basilar cells from adjacent cells and/or underlying dermis (see Figure 9). Clusters of neutrophils were sometimes present within the epidermis and often present in varying numbers within the

microblisters. Neutrophils were present in low numbers and were widely scattered throughout the dermal collagen. Collagen bundles subjacent to the site of application were usually mildly separated by edema fluid, and there were occasional small foci of hemorrhage present in some sections. Hair follicles commonly exhibited epithelial necrosis at their bases but not along the root sheaths. There was a moderate increase in numbers of neutrophils infiltrating the dermis in animals 48 hours post-exposure, as compared to 24 hours post-exposure, but other parameters remained essentially the same.

ELECTRON MICROSCOPY:

The 4 tissue specimens demonstrating the best examples of cleft/vesicle formation on light microscopy were from hairless guinea pigs sacrificed at 24 hours post-exposure and exposed to 0.1, 0.2, 1.0, and 2.0 $\mu$l of HD respectively. Semi-thin section analysis revealed the presence of microblisters at the dermal-epidermal junction (see Figure 10). The floor of the blister cavity was formed by an intact basal lamina in some cases and by remnants of a disrupted basal lamina in others. The upper boundary of the cavity was largely formed by plasmalemma of intact basal cells of the stratum germinativum or by cellular debris of necrotic basal and supra-basal cells of the epidermis. Blister cavities were heavily infiltrated with inflammatory cells, recognized as neutrophils and macrophages, as was the underlying dermis. In most cases, cavities appeared to be interrupted by hair follicles which acted as lateral anchoring demarcation points, while in others, cavities formed above follicles with follicular cells forming part of the floor of the blister. Blisters varied in size, up to 250 $\mu$m in width and up to 80 $\mu$m in height. Early degenerative changes of the basal cell, signalled by paranuclear vacuolation and pyknosis, were most evident at the site of junction between normal and blistered skin. Ultrastructural features of the lesion by thin-section analysis showed the total involvement of basal cells from essentially normal fine-structure at the perimeter of the blister to complete degeneration and necrosis at the centers of the blister (see Figure 11). This progression included perinuclear blebbing, plasmalemmal defects, paranuclear vacuolation, coalescing cytoplasmic vacuoles, pyknotic nuclei, lipid inclusions, lysosomal activity and electron opacity of organelles. Supra-basal cells of the epidermis were also involved to varying degrees especially in central regions of the blister cavity where basal cells were completely degenerated. Invading neutrophils and macrophages, actively phagocytizing degenerating basal cells and other cellular debris were in abundance within cavities. Basal laminae were disrupted and frayed with loosened fibers extending into the blister cavity. Hemidesmosomes, intact at the perimeter of the cavity, were interrupted at the site of the blister with anchoring filaments disabled and free from their attachments to the basal lamina. Within the dermis proper, there was evidence of edema with large displacements of collagen bundles surrounding congested capillaries. Scanning EM showed to advantage and precision the extent, junctions, boundaries, and location of the blister cavities (see Figures 12-13). The three-dimensional nature of the blister, realized only through scanning EM, was useful in determining the relative role of hair follicles in demarcating the limits of some cavities and was especially informative as to the relative size of the blisters.

## DISCUSSION AND CONCLUSIONS

The results of this study indicate that the hairless guinea pig is superior to the haired guinea pig as an animal model for studying HD-induced skin lesions. The hairless guinea pig skin was more sensitive to HD than was haired guinea pig skin at corresponding dose and time points. This increased sensitivity may be due to (1) the hair stubble on the haired animals acting as a physical barrier or (2) fundamental anatomical, biochemical, or immunological differences. There are advantages of the hairless guinea pig: (1) no shaving/clipping is required; (2) lower doses of HD can be used; (3) the resulting gross lesions are more readily visible and easily scored; and (4) the histopathologic lesions were more uniform and contained a higher incidence of microblister formation. A disadvantage of the hairless guinea pig is that they cost 60-70% more per animal.

To properly interpret the gross lesion data it is important to note that neat HD at a constant concentration was used for all exposures and that the delivered dose of HD to the skin was controlled by varying the volume of the droplet that was applied to the skin rather than by varying the concentration of HD in a fixed volume. This explains why we saw large increases in lesion diameters and basically no increases in E/E or E/B scores with increases in doses of HD on the same animal. Lesion diameter was directly proportional to the size/volume of the droplet of neat HD (i.e., the larger the droplet → the larger the area of exposed skin → the larger the resulting lesion). Conversely, the erythema/eschar and edema/blister scores which measure the severity of a lesion are functions of the dose per unit area which was essentially the same for all HD doses (i.e., no change in dose/unit area → no change in lesion severity).

The microscopic skin lesions observed in hairless guinea pig skin were comparable to those observed in pig skin exposed to HD.[6] Both species exhibited histologic response to HD ranging from severe coagulation necrosis to mild basal cell involvement and microblister formation.

The ultrastructural correlates of this study are reminiscent of the ultrastructural pathology of HD-induced blister formation in human skin grafted onto athymic nude mice.[8] Although HD concentrations were not the same, the involvement of the basal cell, the location of the blister at the epidermal-dermal junction and the apparent disabling of the anchoring filaments of the hemidesmosomes are unequivocal. A persistent difference between the two studies is the pronounced infiltration of inflammatory cells in this study, which although noted within the previous study, was not to the same degree.

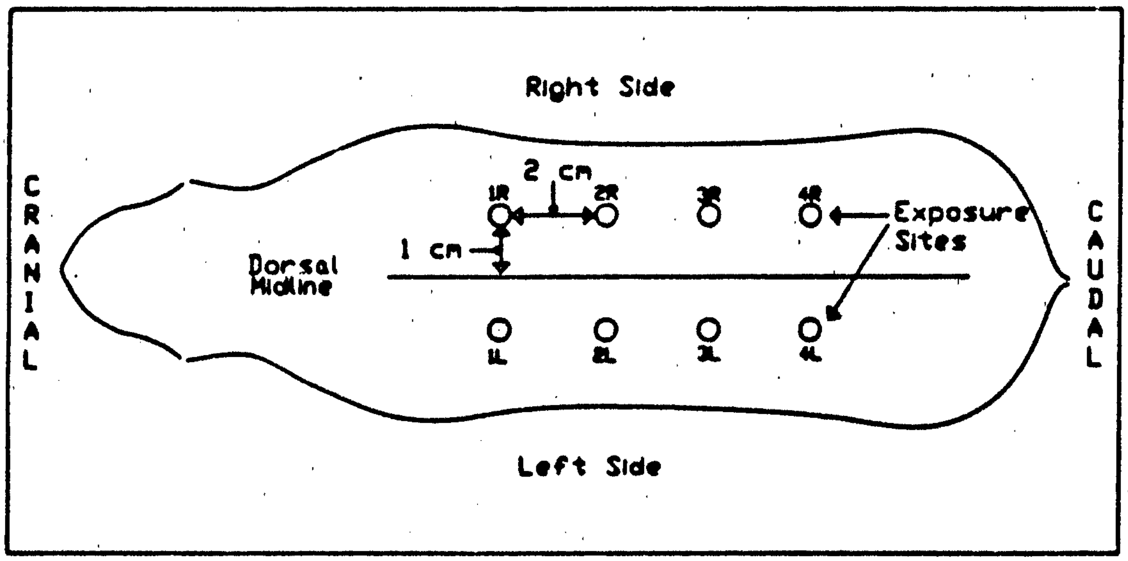


**Figure 1:** Guinea pig skin exposure sites (dorsal view).

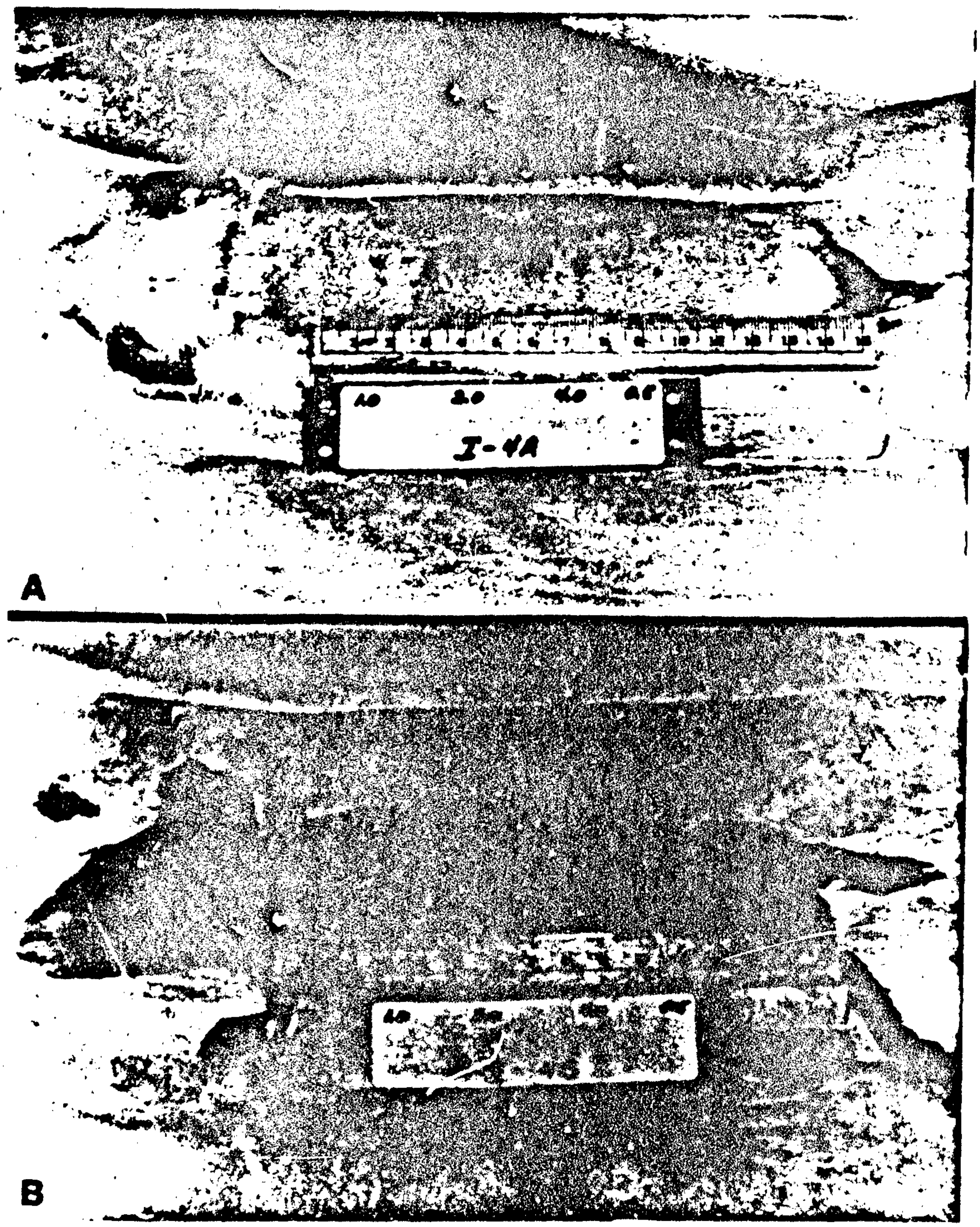


Figure 2: Gross skin lesions in haired (A) vs. hairless (B) guinea pigs 24 hours after exposure to 0.5-4.0 µl of sulfur mustard (HD).

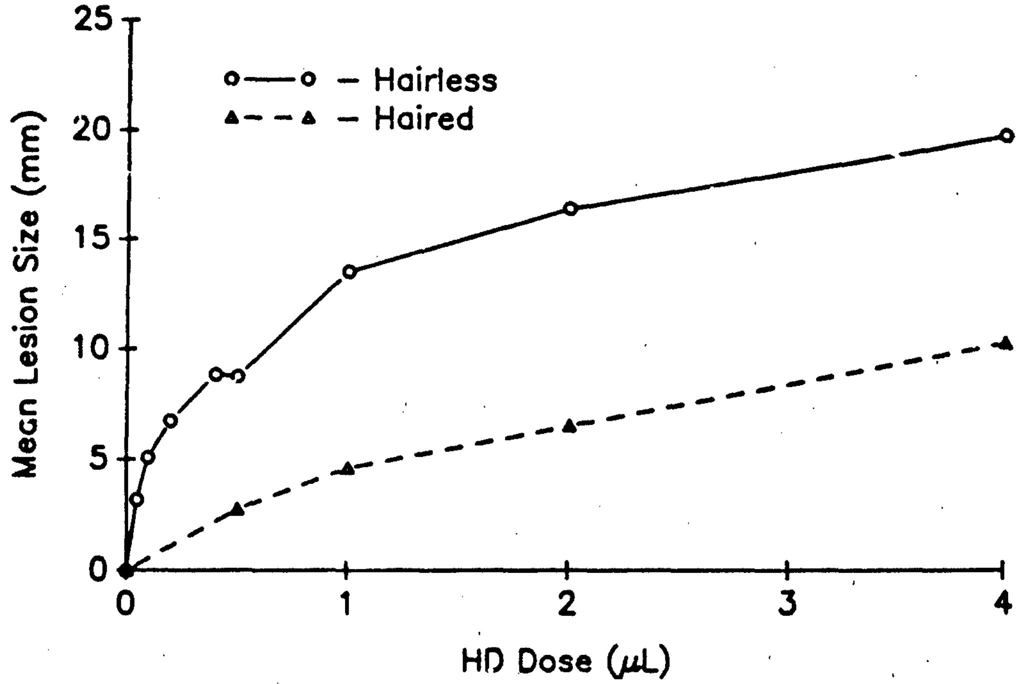


**Figure 3:** Mean skin lesion size in haired vs. hairless guinea pigs 24 hours after exposure to sulfur mustard (HD).

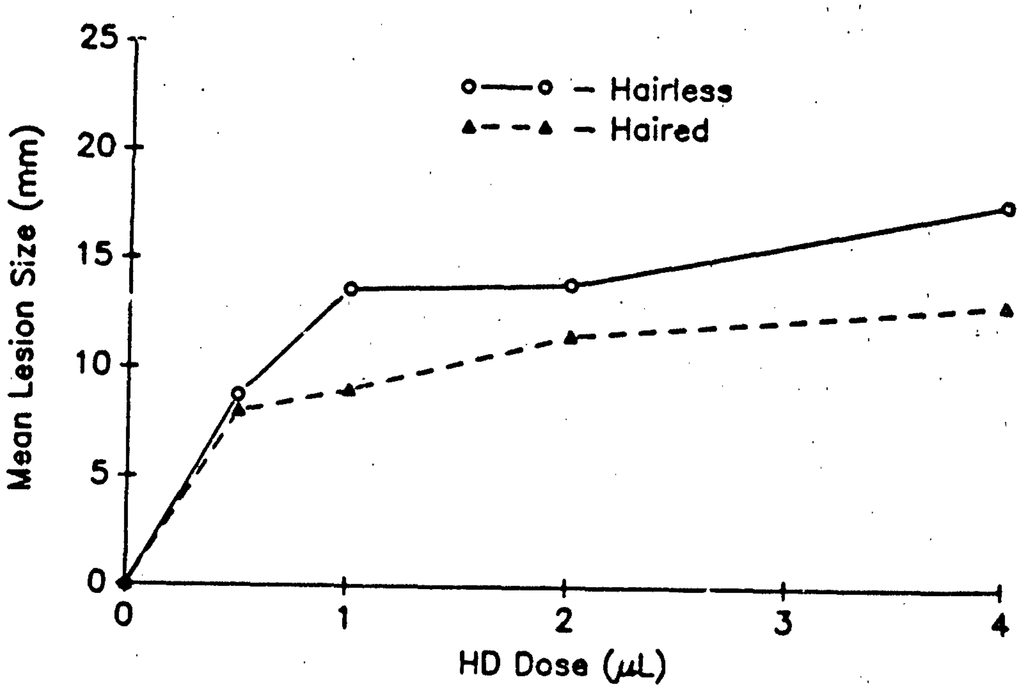


**Figure 4:** Mean skin lesion size in haired vs. hairless guinea pigs 48 hours after exposure to sulfur mustard (HD).

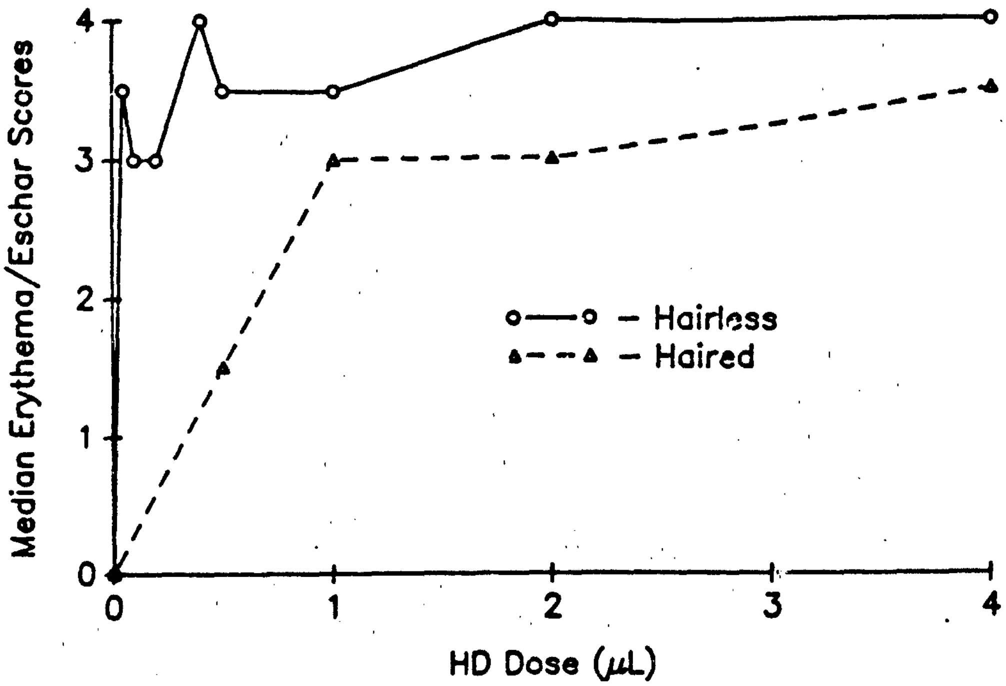


Figure 5: Median erythema/eschar scores in haired vs. hairless guinea pigs 24 hours after exposure to sulfur mustard (HD).

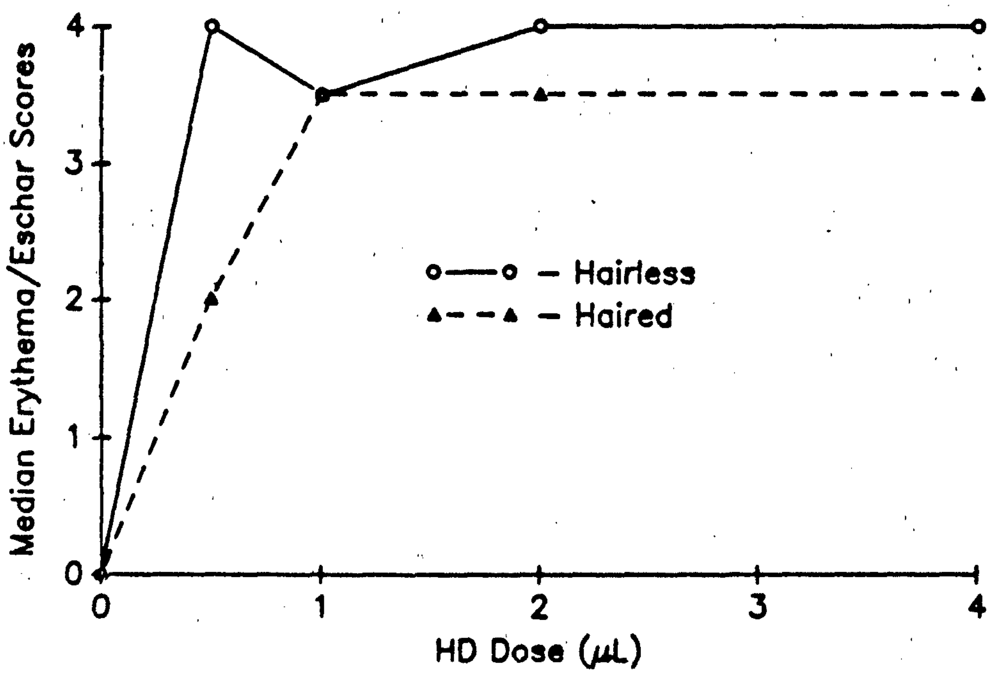


Figure 6: Median erythema/eschar scores in haired vs. hairless guinea pigs 48 hours after exposure to sulfur mustard (HD).

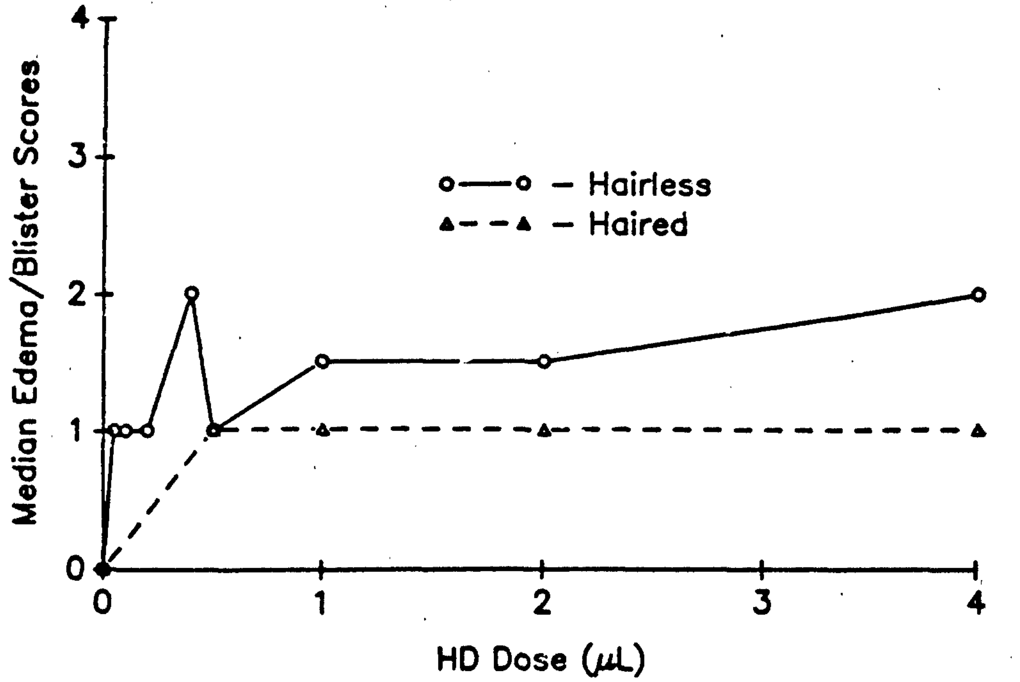


**Figure 7:** Median edema/blister scores in haired vs. hairless guinea pigs 24 hours after exposure to sulfur mustard (HD).

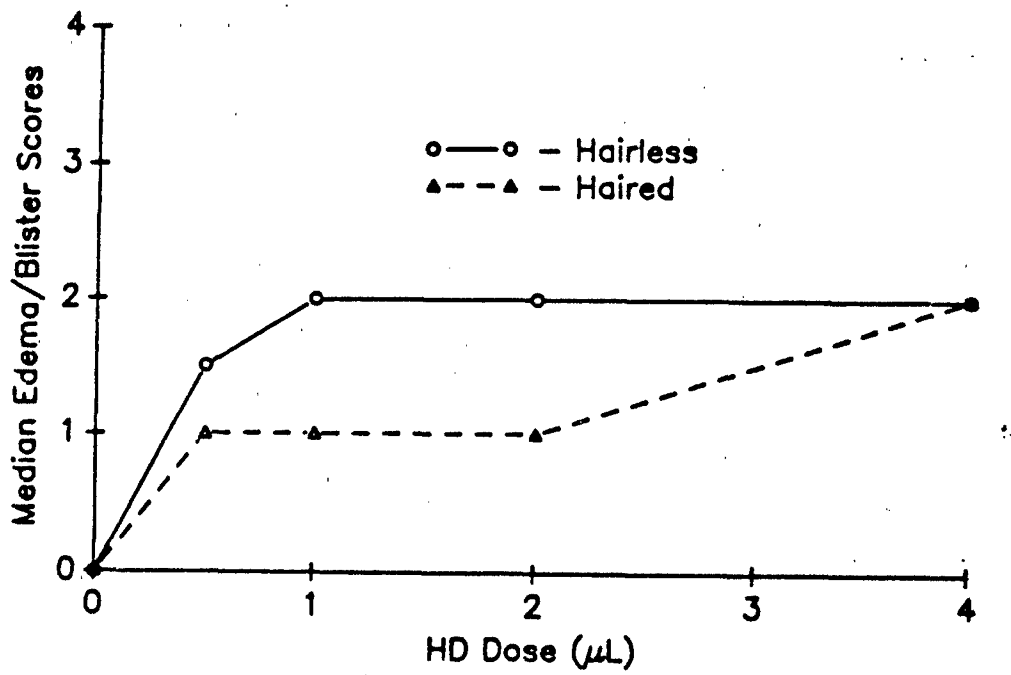


**Figure 8:** Median edema/blister scores in haired vs. hairless guinea pigs 48 hours after exposure to sulfur mustard (HD).

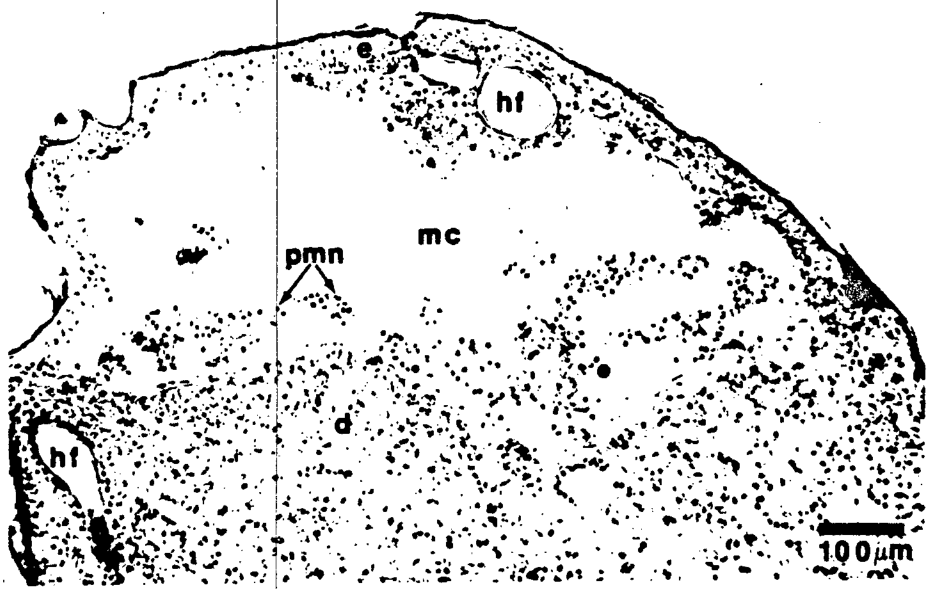


Figure 9: Microblister in hairless guinea pig skin at 24 hours after exposure to 0.2 µl of sulfur mustard (HD). Microblister cavity (mc), epidermis (e), dermis (d), hair follicles (hf), and polymorphonuclear cells (pmn). H&E.

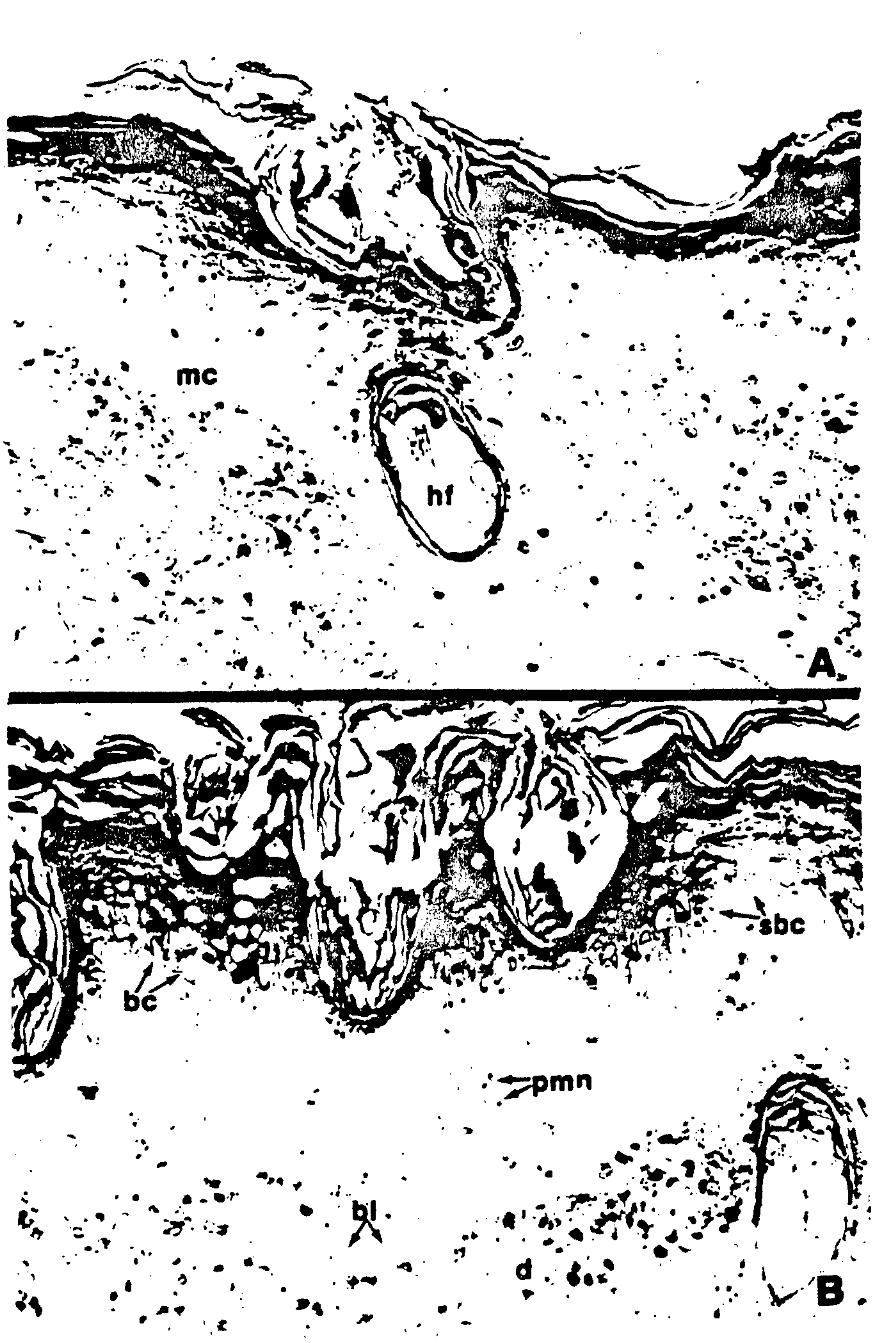


Figure 10: Semithin epoxy sections through centers of microblisters at the dermal-epidermal junction of hairless guinea pig skin. A. Microblister cavity (mc) interrupted by a hair follicle (hf). (330X) B. Microblister cavity infiltrated with polymorphonuclear leukocytes (pmn) and filled with cellular debris. The roof of the cavity is formed by basal cells (bc) and suprabasal cells (sbc) of the epidermis with the floor formed by the basla lamina (bl) of the dermis (d). (330X) Humphrey's stain.

Figure 11: Transmission electron micrographs of microblister cavity formation at the dermal-epidermal junction of the hairless guinea pig. A. At the perimeter of the blister cavity, basal cells (bc) of the stratum germinativum show progressive subcellular changes signalled by nuclear condensation of chromatin (nc), blebbing of the perinuclear envelope (pb), and paranuclear vacuolation (v). (12,000X) B. Area at the perimeter showing disabling of anchoring filaments (af) of the basal cell hemidesmosomes (hd). (30,000X) C. The cavity, infiltrated with neutrophils (ne) and cellular detritus, is demarcated by the basla lamina (bl) of the dermis (d) and degenerating basal cells and supra basla cells of the epidermis (epi). (9,000X)


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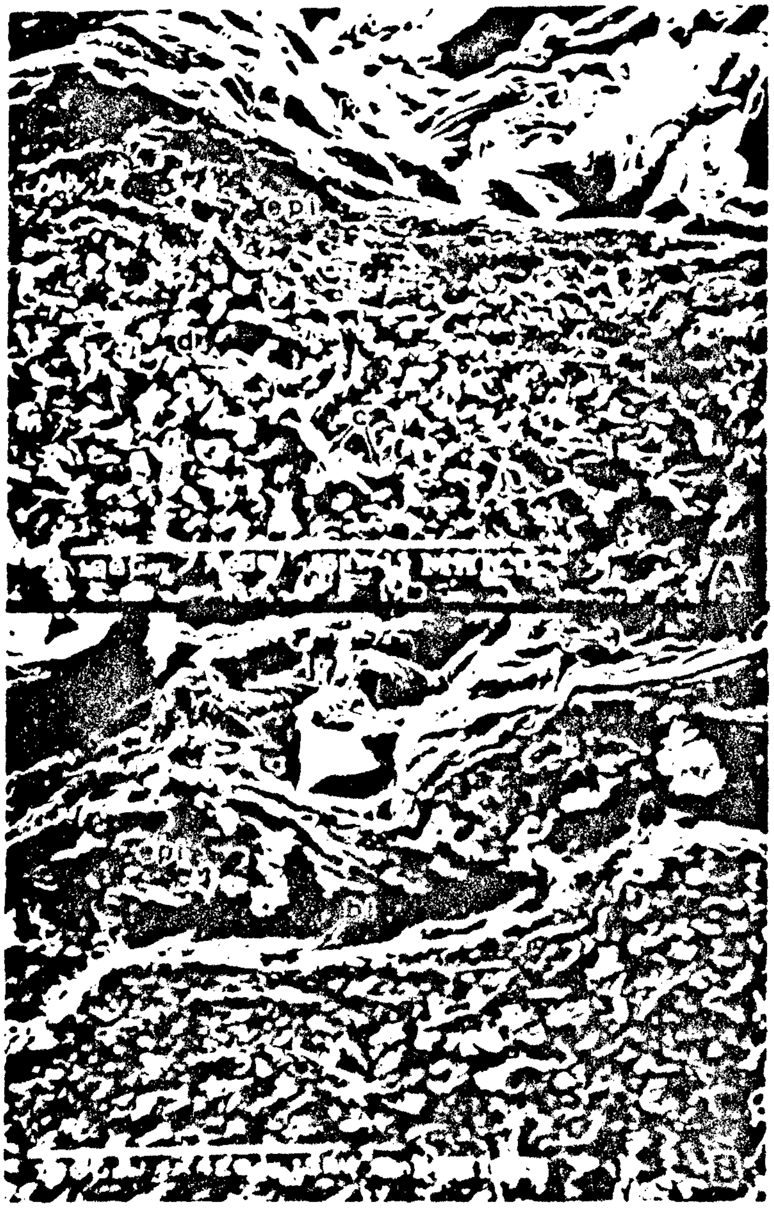


Figure 12: Scanning electron micrographs of the dermal-epidermal junction of hairless guinea pig skin. A. Area of non-blistered skin: epidermis (epi); dermis (d); keratin (k); and collagen fibers (c). B. Area of microblister formation. Microblister cavity is bordered by cells of the epidermis at the roof and the basal lamina (bl) at the floor.

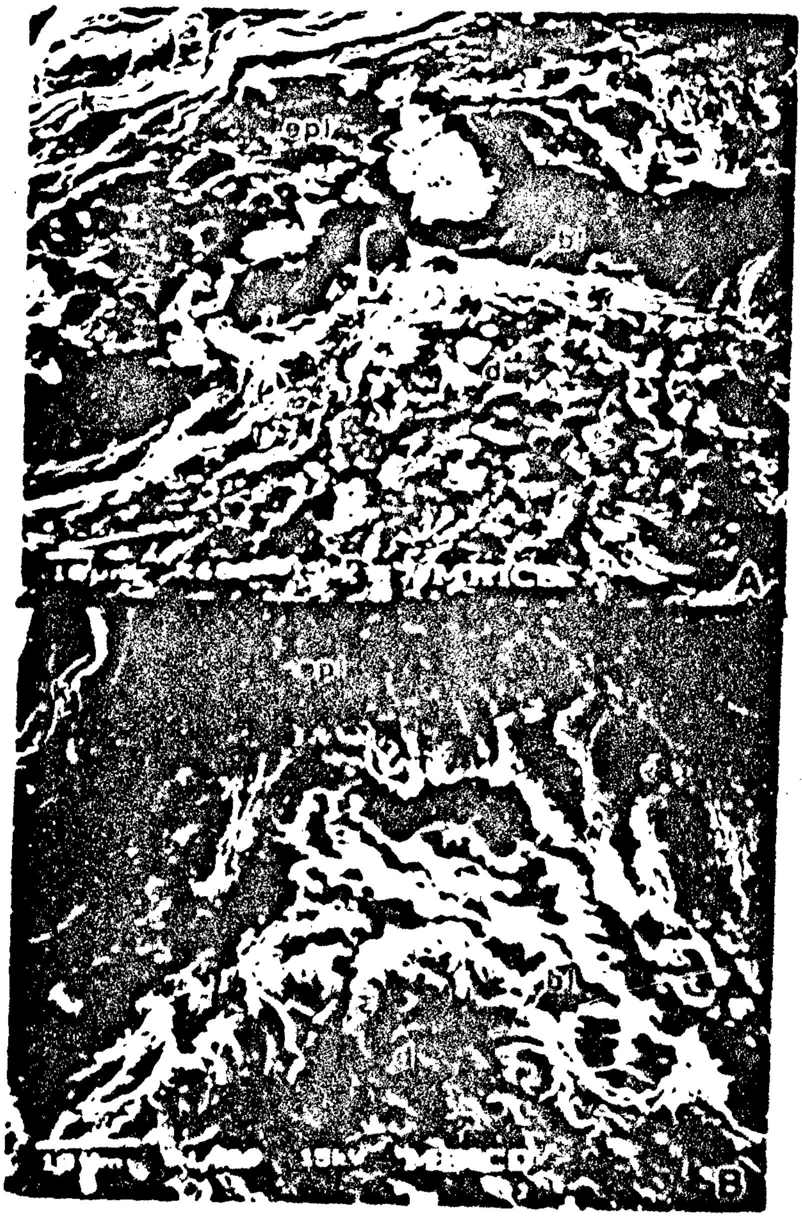


Figure 13: Higher magnification scanning electron micrographs through centers of microblisters. A & B. The boundaries and the three dimensional nature of the blister cavities are clearly visible. Cavities vary in size, up to 250 $\mu m$ in width and up to 80 $\mu m$ in height. Legend: epidermis (epi); dermis (d); basal lamina (bl); and keratin (k).

TABLE 1: EXPERIMENTAL DESIGN

| | NO. HAIRED | NO. HAIRLESS | DOSE RANGE | TIME |
|---|---|---|---|---|
| GROUP I | 4 | 4 | 0.5-4.0 ul | 24 hrs |
| GROUP II | 4 | 4 | 0.5-4.0 ul | 48 hrs |
| GROUP III | 0 | 3 | 0.05-0.4 ul | 24 hrs |

TABLE 2: DOSES OF SULFUR MUSTARD (HD) APPLIED TO HAIRED AND HAIRLESS GUINEA PIG SKIN

| ANIMAL NUMBER | SITE 1L | SITE 2L | SITE 3L | SITE 4L | SITE 1R | SITE 2R | SITE 3R | SITE 4R |
|---|---|---|---|---|---|---|---|---|
| I-1A | 0.5 | 1.0 | 2.0 | 4.0 | 0 | 0 | 0 | 0 |
| I-1B | 0.5 | 1.0 | 2.0 | 4.0 | 0 | 0 | 0 | 0 |
| I-2A | 4.0 | 0.5 | 1.0 | 2.0 | 0 | 0 | 0 | 0 |
| I-2B | 4.0 | 0.5 | 1.0 | 2.0 | 0 | 0 | 0 | 0 |
| I-3A | 2.0 | 4.0 | 0.5 | 1.0 | 0 | 0 | 0 | 0 |
| I-3B | 2.0 | 4.0 | 0.5 | 1.0 | 0 | 0 | 0 | 0 |
| I-4A | 1.0 | 2.0 | 4.0 | 0.5 | 0 | 0 | 0 | 0 |
| I-4B | 1.0 | 2.0 | 4.0 | 0.5 | 0 | 0 | 0 | 0 |
| II-1A | 0.5 | 1.0 | 2.0 | 4.0 | 0 | 0 | 0 | 0 |
| II-1B | 0.5 | 1.0 | 2.0 | 4.0 | 0 | 0 | 0 | 0 |
| II-2A | 4.0 | 0.5 | 1.0 | 2.0 | 0 | 0 | 0 | 0 |
| II-2B | 4.0 | 0.5 | 1.0 | 2.0 | 0 | 0 | 0 | 0 |
| II-3A | 2.0 | 4.0 | 0.5 | 1.0 | 0 | 0 | 0 | 0 |
| II-3B | 2.0 | 4.0 | 0.5 | 1.0 | 0 | 0 | 0 | 0 |
| II-4A | 1.0 | 2.0 | 4.0 | 0.5 | 0 | 0 | 0 | 0 |
| II-4B | 1.0 | 2.0 | 4.0 | 0.5 | 0 | 0 | 0 | 0 |
| III-1B | 0.05 | 0.1 | 0.2 | 0.4 | 0.4 | 0.2 | 0.1 | 0.05 |
| III-2B | 0.4 | 0.05 | 0.1 | 0.2 | 0.2 | 0.1 | 0.05 | 0.4 |
| III-3B | 0.2 | 0.4 | 0.05 | 0.1 | 0.1 | 0.05 | 0.4 | 0.2 |

Footnotes:
1. Dose of HD is expressed in microliters (ul)
2. Animal number alfanumeric code: Roman Numeral = Experimental group; Arabic Numeral = Individual/pair designator within an experimental group; A = Haired; B = Hairless.
3. Site code: Arabic numeral = site numbered from anterior to posterior; L = Left; R = Right.

TABLE 3: MEAN GROSS LESION DIAMETER, MEDIAN ERYTHEMA/ESCHAR SCORE, AND MEDIAN EDEMA/BLISTER SCORE AT 24 AND 48 HOURS POST-EXPOSURE IN HAIRED GUINEA PIGS.

| HD DOSE (ul) | 24 HOURS POST-EXPOSURE | | | 48 HOURS POST-EXPOSURE | | |
|---|---|---|---|---|---|---|
| | DIAMETER (mm) | ERYTHEMA/ ESCHAR SCORE | EDEMA/ BLISTER SCORE | DIAMETER (mm) | ERYTHEMA/ ESCHAR SCORE | EDEMA/ BLISTER SCORE |
| 0.5 | 2.75 | 1.50 | 1.00 | 8.00 | 2.00 | 1.00 |
| 1.0 | 4.60 | 3.00 | 1.00 | 9.00 | 3.50 | 1.00 |
| 2.0 | 6.50 | 3.00 | 1.00 | 11.50 | 3.50 | 1.00 |
| 4.0 | 10.25 | 3.50 | 1.00 | 13.00 | 3.50 | 2.00 |

| ERYTHEMA/ESCHAR SCORE: | EDEMA/BLISTER SCORE: |
|---|---|
| No erythema......................0 | No edema.........................0 |
| Slight erythema (barely seen)....1 | Slight edema (barely seen).......1 |
| Moderate erythema (pink).........2 | Moderate edema (well defined)....2 |
| Severe erythema (beet red).......3 | Severe edema (raised >1 mm)......3 |
| Eschar formation (necrosis)......4 | Blister formation................4 |

TABLE 4: MEAN GROSS LESION DIAMETER, MEDIAN ERYTHEMA/ESCHAR SCORE, AND MEDIAN EDEMA/BLISTER SCORE AT 24 AND 48 HOURS POST-EXPOSURE IN HAIRLESS GUINEA PIGS.

| HD DOSE (ul) | 24 HOURS POST-EXPOSURE | | | 48 HOURS POST-EXPOSURE | | |
|---|---|---|---|---|---|---|
| | DIAMETER (mm) | ERYTHEMA/ ESCHAR SCORE | EDEMA/ BLISTER SCORE | DIAMETER (mm) | ERYTHEMA/ ESCHAR SCORE | EDEMA/ BLISTER SCORE |
| 0.05 | 3.17 | 3.50 | 1.00 | n/a | n/a | n/a |
| 0.1 | 5.08 | 3.00 | 1.00 | n/a | n/a | n/a |
| 0.2 | 6.75 | 3.00 | 1.00 | n/a | n/a | n/a |
| 0.4 | 8.83 | 4.00 | 2.00 | n/a | n/a | n/a |
| 0.5 | 8.75 | 3.50 | 1.00 | 8.75 | 4.00 | 1.50 |
| 1.0 | 13.50 | 3.50 | 1.50 | 13.62 | 3.50 | 2.00 |
| 2.0 | 16.38 | 4.00 | 1.50 | 13.88 | 4.00 | 2.00 |
| 4.0 | 19.75 | 4.00 | 2.00 | 17.62 | 4.00 | 2.00 |

| ERYTHEMA/ESCHAR SCORE: | EDEMA/BLISTER SCORE: |
|---|---|
| No erythema......................0 | No edema.........................0 |
| Slight erythema (barely seen)....1 | Slight edema (barely seen).......1 |
| Moderate erythema (pink).........2 | Moderate edema (well defined)....2 |
| Severe erythema (beet red).......3 | Severe edema (raised >1 mm)......3 |
| Eschar formation (necrosis)......4 | Blister formation................4 |

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## APPENDIX A

| EXPERIMENTAL RECORD/SCHEDULE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ANIMAL NUMBER | ANESTHESIA | | | | | SULFUR MUSTARD (HD) CHALLENGE | | | | | |
| | Ketamine (100 mg/ml): 30 mg/kg IM<br>Rompum (20 mg/ml): 6 mg/kg IM | | | | | HD DOSE/SKIN SITE | | | | EXPOSURE TIME | |
| | WEIGHT (grams) | KETAMINE (cc) | ROMPUM (cc) | START TIME | END TIME | SITE 1L | SITE 2L | SITE 3L | SITE 4L | START TIME | END TIME |
| I-1A | | | | | | 0.5 | 1.0 | 2.0 | 4.0 | | |
| I-1B | | | | | | 0.5 | 1.0 | 2.0 | 4.0 | | |
| I-2A | | | | | | 4.0 | 0.5 | 1.0 | 2.0 | | |
| I-2B | | | | | | 4.0 | 0.5 | 1.0 | 2.0 | | |
| I-3A | | | | | | 2.0 | 4.0 | 0.5 | 1.0 | | |
| I-3B | | | | | | 2.0 | 4.0 | 0.5 | 1.0 | | |
| I-4A | | | | | | 1.0 | 2.0 | 4.0 | 0.5 | | |
| I-4B | | | | | | 1.0 | 2.0 | 4.0 | 0.5 | | |
| II-1A | | | | | | 0.5 | 1.0 | 2.0 | 4.0 | | |
| II-1B | | | | | | 0.5 | 1.0 | 2.0 | 4.0 | | |
| II-2A | | | | | | 4.0 | 0.5 | 1.0 | 2.0 | | |
| II-2B | | | | | | 4.0 | 0.5 | 1.0 | 2.0 | | |
| II-3A | | | | | | 2.0 | 4.0 | 0.5 | 1.0 | | |
| II-3B | | | | | | 2.0 | 4.0 | 0.5 | 1.0 | | |
| II-4A | | | | | | 1.0 | 2.0 | 4.0 | 0.5 | | |
| II-4B | | | | | | 1.0 | 2.0 | 4.0 | 0.5 | | |
| | | | | | | | | | | | |

## APPENDIX A
(cont.)

EXPERIMENTAL RECORD/SCHEDULE

| ANIMAL NUMBER | ANESTHESIA: Ketamine (100 mg/ml): 30 mg/kg IM; Rompun (20 mg/ml): 6 mg/kg IM | | | | | SULFUR MUSTARD (HD) CHALLENGE: HD DOSE/SKIN SITE | | | | EXPOSURE TIME | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | WEIGHT (grams) | KETAMINE (cc) | ROMPUM (cc) | START TIME | END TIME | SITE 1 L&R | SITE 2 L&R | SITE 3 L&R | SITE 4 L&R | START TIME | END TIME |
| III-1B | | | | | | .05/L | .1/L | .2/L | .4/L | | |
| | | | | | | .4/R | .2/R | .1/R | .05/R | | |
| III-2B | | | | | | .4/L | .05/L | .1/L | .2/L | | |
| | | | | | | .2/R | .1/R | .05/R | .4/R | | |
| III-3B | | | | | | .2/L | .4/L | .05/L | .1/L | | |
| | | | | | | .1/R | .05/R | .4/R | .2/R | | |

COMMENTS:

COMMENTS:

## APPENDIX B

| GROSS LESION WORKSHEET | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HD DOSE: 0.5 ul<br>TIME: 24 hrs | | | HAIRED GUINEA PIGS | | | | | | HAIRLESS GUINEA PIGS | | | | | |
| | | | LEFT SIDE | | | RIGHT SIDE | | | LEFT SIDE | | | RIGHT SIDE | | |
| GROUP | PAIR | SITE | SIZE | E/E | E/B | SIZE | E/E | E/B | SIZE | E/E | E/B | SIZE | E/E | E/B |
| I | 1 | 1 | | | | | | | | | | | | |
| I | 2 | 2 | | | | | | | | | | | | |
| I | 3 | 3 | | | | | | | | | | | | |
| I | 4 | 4 | | | | | | | | | | | | |
| TOTAL | | | | | | | | | | | | | | |
| AVERAGE | | | | | | | | | | | | | | |
| HD DOSE: 0.5 ul<br>TIME: 48 hrs | | | | | | | | | | | | | | |
| II | 1 | 1 | | | | | | | | | | | | |
| II | 2 | 2 | | | | | | | | | | | | |
| II | 3 | 3 | | | | | | | | | | | | |
| II | 4 | 4 | | | | | | | | | | | | |
| TOTAL | | | | | | | | | | | | | | |
| AVERAGE | | | | | | | | | | | | | | |
| SIZE:<br>Diameter of the lesion measured in millimeters (mm). | | | ERYTHEMA/ESCHAR SCORE: (E/E)<br>No erythema 0<br>Slight erythema (barely seen) 1<br>Moderate erythema (pink) 2<br>Severe erythema (beet red) 3<br>Eschar formation (necrosis/sloughing) 4 | | | | | | EDEMA/BLISTER SCORE: (E/B)<br>No edema 0<br>Slight edema (barely seen) 1<br>Moderate edema (well defined) 2<br>Severe edema (raised >1 mm) 3<br>Blister formation 4 | | | | | |

## APPENDIX B
(cont.)

### GROSS LESION WORKSHEET

| DOSE: .05 ul HD | | 24 HOURS POST-EXPOSURE | | | |
|---|---|---|---|---|---|
| ANIMAL NO. | SITE | SIZE | E/E | E/B | NOTES |
| III-1B | 1L | | | | |
| III-1B | 4R | | | | |
| III-2B | 2L | | | | |
| III-2B | 3R | | | | |
| III-3B | 3L | | | | |
| III-3B | 2R | | | | |
| TOTAL | | | | | |
| AVERAGE | | | | | |
| DOSE: 0.1 ul HD | | 24 HOURS POST-EXPOSURE | | | |
| ANIMAL NO. | SITE | SIZE | E/E | E/B | NOTES |
| III-1B | 2L | | | | |
| III-1B | 3R | | | | |
| III-2B | 3L | | | | |
| III-2B | 2R | | | | |
| III-3B | 4L | | | | |
| III-3B | 1R | | | | |
| TOTAL | | | | | |
| AVERAGE | | | | | |

**SCORING SYSTEM**

**SIZE:**

Diameter of the lesion measured in millimeters (mm) from the outermost margin of visible erythema.

**ERYTHEMA/ESCHAR SCORE: (E/E)**

No erythema....................0
Slight erythema (barely seen)..1
Moderate erythema (pink).......2
Severe erythema (beet red).....3
Eschar (necrosis/sloughing)....4

**EDEMA/BLISTER SCORE: (E/B)**

No edema.......................0
Slight edema (barely seen).....1
Moderate edema (well defined)..2
Severe edema (raised >1 mm)....3
Blister........................4

## APPENDIX C

DERMAL HISTOPATHOLOGY WORKSHEET

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ACCESSION NO. | | BLOCK NO. | 1 | 2 | 3 | 4 | | 5 | 6 | 7 | 8 |
| ANIMAL I.D. NO. | | SITE NO. | 1L | 2L | 3L | 4L | | 1R | 2R | 3R | 4R |
| LESION PRESENT (i.e., necrosis, vesicle, etc.) (+/-) | | | | | | | | | | | |
| HORIZONTAL DIST. (mm) | | | | | | | | | | | |
| LESION DEPTH (mm) (i.e., depth of necrosis) | | | | | | | | | | | |
| EPIDERMOLYSIS (+/-) | | | | | | | | | | | |
| ACANTHOLYSIS (+/-) | | | | | | | | | | | |
| PUSTULAR EPIDERMATITIS (+/-) | | | | | | | | | | | |
| VESICLE/CLEFT (+/-) | | | | | | | | | | | |
| DERMAL EDEMA (+/-) | | | | | | | | | | | |
| DERMAL CONGESTION (+/-) | | | | | | | | | | | |
| PUSTULAR DERMATITIS (+/-) | | | | | | | | | | | |
| PERIVASCULITIS (+/-) | | | | | | | | | | | |
| FOLLICULAR INVOLVEMENT (i.e., necrosis)(+/-) | | | | | | | | | | | |
| | | | | | | | | | | | |
| | | | | | | | | | | | |
| | | | | | | | | | | | |
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